This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 21!

See attached form for additional information.

NOV 29 2005

Interagency Report Control No.:

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

1. CERTIFICATE NUMBER: 51-F-0003
CUSTOMER NUMBER: 443

FORM APPROVED
OMB NO. 0579-0036

Armed Forces Radiobiology Research Inst.
Afrri/Vsd
8901 Wisconsin Avenue
Bldg 42
Bethesda, MD 20889

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, or experimentation, or held for these purposes. Attach additional sheets if necessary)

FACILITY LOCATIONS (Sites) - See Atached Listing

All animals located on-site

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY (Attach additional sheets if necessary or use APHIS Form 7023A)

A. Animals Covered By The Animal Welfare Regulations	B. Number of animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals an for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reast such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs					
7. Hamsters					
8. Rabbits					
9. Non-human Primates			63	20	83
10. Sheep					
11. Pigs				15	15
12. Other Farm Animals					
13. Other Animals					
Mice	1117	4036	413	10077	14526

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anestetic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and ap Institutional Animal Care and Use Committee (IACUC). A summary of all such exceptions is attached to this annual report. In addition to identifying the IACUC-approved exceptions, this summary inc brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional Official)

SI

(b)(6), (6)(7)(C)

NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print)

(b)(6), (6)(7)(C)

DATE SIGNED
23 Nov 05

AP

Category E

1010mice

The research questions that we are addressing involve complex interactions between many tissues that would be affected by the administration of drugs to alleviate pain. The pain and distress experienced by these animals would probably be similar to what humans experience during severe cold and flu infections. Radiation itself has been demonstrated to reduce pain in laboratory mice (Teskey and Kavaliers 1984; Seong et al, 2004) and has been reported to alleviate cancer related bone pain in humans (Ciezki and Macklis 1995; Sonoo et al. 1995). The isoflavones used in these experiments have been demonstrated to protect mice from radiation injury and also to enhance the immune system and reduce pain (Shir et al. 2002; Liu et al., 2004) and inflammation (Verdrengh et al., 2003), and will likely reduce any radiation-induced discomfort. Because of the complexity of physiological responses that occurs after radiation exposure, and our poor understanding of its induction and progression, there is no other way, at present, to evaluate the effectiveness of radiation protectants to enhance survival of humans than to do these experiments in animals. One of the aims of the present study is to acquire an understanding of cellular and molecular correlates of radioprotection in order to develop techniques to evaluate these compounds with less reliance on animal studies.

Ciezki J, Macklis RM: The palliative role of radiotherapy in the management of the cancer patient. Semin Oncol 1995;22:82-90.

Liu L, Yang T, Simon SA The protein tyrosine kinase inhibitor, genistein, decreases excitability of nociceptive neurons. Pain. 2004 Nov;112(1-2):131-41.

Shir Y, Campbell JN, Raja SN, Seltzer Z: The correlation between dietary soy phytoestrogens and neuropathic pain behavior in rats after partial denervation. Anesth Analg 2002;94:421-426.

Sonoo H, Shimozuma K, Kurebayashi J, Ohta K, Kiyono T: Systemic therapy, pain relief and quality of life of breast cancer patients with bone metastasis. Gan To Kagaku Ryoho 1995;22 Suppl 1:10-15.

Teskey GC, Kavaliers M: Ionizing radiation induces opioid-mediated analgesia in male mice. Life Sci 1984;35:1547-1552.

Verdrengh, M., Collins, L. V., Bergin, P., & Tarkowski, A. (2004). Phytoestrogen genistein as an anti-staphylococcal agent. Microbes Infect., 6, 86-92.

1087 mice

Painful Procedure Justification:
There are no alternative procedures for irradiation because it is a unique stimulus that cannot be otherwise duplicated. Radiation itself does not cause pain or distress. In fact, radiation can alleviate the pain associated with cancer (Bateman, 1994; Ciezki and Macklis, 1995; Page, 1995; Sonoo et al., 1995; Thrall, 1995). Nevertheless, the sequelae of nausea, vomiting, and diarrhea cause pain and distress in humans in the early post-irradiation period, when lethal doses are used. However, mice are not susceptible to vomiting. Although radiation does not induce pain, animals in these experiments might experience pain and discomfort prior to death because of sequelae. To avoid possibly affecting survival/death outcomes, and ultimately $LD_{50/30}$ and $LD_{95/30}$ calculations, analgesics/sedatives will not be administered after challenge so as not to interfere with the clinical course of the infectious disease. The attending veterinarian was consulted with respect to the procedures described above, when they were used on previous protocols.

These studies are designed to assess the susceptibility and immune response to the combined effects of ionizing radiation and bacterial infection. There are no alternatives to the use of animals in these studies. Similarly, there are no alternatives to bacterial challenge or natural, radiation-induced infection, because protective immunity cannot be predicted from seroconversion alone at this time when it occurs. Moribund animals will be euthanized as indicated to alleviate further pain and distress.

Bacteria cause infections that cause discomfort either locally or systemically. Many pathogenic bacteria have unusual or even unique virulent characteristics, but they also have common attributes, including binary multiplication and penetration of tissues, and cause common responses and disease processes in animals, which cannot be mimicked readily by substitutes. Although, by necessity, there are animals included in the unalleviated pain-and-distress category in this protocol, there will be a conscious effort by the P.I. and animal care staff to provide as much additional consideration for the comfort and well-being of the animals as is consistent with the scientific integrity of the study.

Although we expect that test therapeutic agents will provide some relief to some of the mice, an alternative for these procedures would be to determine whether an analgesic could be used to relieve pain and discomfort. Although the opioid analgesics, butorphanol and bupenorphine, might be used to alleviate local pain associated with a local infection without altering the local inflammatory response (Swearengen et al. 1993), opioid analgesics are immunomodulatory (Pruett et al. 1992, Pasotti et al. 1993, Carr et al. 1994). Other, non-narcotic analgesics, such as indomethacin, are anti-inflammatory, so they would interfere with the inflammatory responses of the hemopoietic tissues to infections. Analgesics cause adverse effects on undamaged hematopoietic cells (Hollaender, 1960) and

interfere with nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, a key polymorphonuclear leukocyte enzyme that is involved in the ability of these cells to phagocytize and kill bacteria (Moon et al., 1986).

REFERENCES (Painful Procedure Justification):

Bateman, K.E. 1994. 0-7-21 radiation therapy for the palliation of advanced cancer in dogs. J. vet. intern. med. 8: 394-399.

Carr, D. J., Gerak, L. R., and France, C. P. 1994. Maltrexone antagonizes the analgesic and immunosuppressive effects of morphine in mice. J. Pharmacol. Exp. Ther. 269(2):693-698.

Ciezki, J., and Macklis, R. M. 1995. The palliative role of radiotherapy in the management of the cancer patient. Semin. Oncol. 22(2, Suppl 3):82-90.

Hollaender, A., ed. 1960. Radiation protection and recovery. Pergamon Press, London.

Moon, B. C., M. J. Girotti, S. G. F. Wren, R. Dawson, and D. Brar. 1986. Effect of antibiotics and sedatives on normal neutrophil nicotinamide adenine dinucleotide phosphate-reduced oxidase activity. Arch. Surg. 121:73-76.

Page, R. L. 1995. Radiation therapy in the management of pain associated with cancer. Proc. North Am. Vet. Conf. Jan. 14-19, 1995, Orlando, Florida. p. 258.

Pasotti, D., Mazzone, A., Lecchini, S., Frigo, G. M., and Ricevuti, G. 1993. Influenza dei peptidi oppioidi sui granulociti del sangue periferico. [The effect of opioid peptides on peripheral blood granulocytes.] Riv. Eur. Sci. Med. Farmacol. 15(2):71-81.

Pruett, S. B., Han, Y. C., and Fuchs, B. A. 1992. Morphine suppresses primary humoral immune responses by a predominantly indirect mechanism. J. Pharmacol. Exp. Ther. 262(3):923-928.

Sonoo, H., Shimozuma, K., Kurebayashi, J., Ohta, K., and Kiyono, T. 1995. [Systemic therapy, pain relief and quality of life of breast cancer patients with bone metastasis.] Gan To Kagaku Ryoho 22 Suppl 1:10-15.

Swearengen, J. R., Cockman-Thomas R. A., Davis, J. A., and P. J. Weiss. 1993. Evaluation of butophanol tartrate and bupenorphine hydrochloride on the inflammatory reaction of the Sereny Test. Lab. Animal Sci. 43(5):417-525.

Thrall, D.E. 1995. Palliative radiation therapy. Sem. vet. med. surg. (small anim.) (special issue on Radiation oncology edited by E. L. Gillette)10:205-208.

1044 mice

Since the purpose of our study was to determine the effects of radiation and countermeasure agents on the functions of the immune system, we did not use analgesics and anesthetics. Alterations of the immune system by analgesics and anesthetics have been documented in literature and have been referred to in the IACUC protocol. However, animals used for blood collection were anesthetized appropriately prior to blood sample collection.

1580 mice

Since the purpose of our study was to determine the effects of radiation and countermeasure agents on the functions of the immune system, we did not use analgesics and anesthetics. Alterations of the immune system by analgesics and anesthetics have been documented in literature and have been referred to in the IACUC protocol. However, animals used for blood collection were anesthetized appropriately prior to blood sample collection.

768 mice

As described in the protocol sections on "Non-animal Alternatives considered" and "Anesthesia/ Analgesia/Tranquilization", the research questions that we are attempting to address involve complex interactions between different tissues that would be affected by the administration of drugs to alleviate pain. As discussed above with references to the literature search on alternatives to painful procedures, the pain associated with the radiation experiments derives from the fact that the animals succumb to infections because of compromised immune systems. The pain and distress experienced by these animals would probably be similar to what we experience during severe cold and flu infections. Because of the physiological complexity of radiation injury, and our lack of having a full understanding of its induction and progression, there is just no other way to evaluate the effectiveness of radioprotectants designed to enhance survival than to do survival experiments using intact animals. One of the aims of the present proposal is to provide the foundation for further studies designed to extend our understanding of cellular and molecular correlates of radioprotection in order to develop ways of evaluating radioprotectors in future with less reliance on survival studies.

15 Pigs

Human contact, socialization, acclimatization to restraint devices, enrichment, and nursing care were used to address pain and distress in non-pharmacological ways. However, all animals, to include controls, had unexpected infectious complications leading to some potential inflammatory pain that was not relieved due to the attending veterinarian's belief that analgesics would interfere with the immune and inflammatory responses and subsequent data collection.

20 Macaca mulatta.

Pain category E, Justification was in the approved IACUC protocol and remains the same. All animals were irradiated with 6.5 Gy whole-body irradiation and provided ciprofloxacin management subsequent to the dosing. The act of irradiation does not induce pain but rather, the sequelae of radiation-induced sickness are scored as painful. The dose of radiation was designed to induce lethality but due to the excellent management by all staff members all irradiated animals survived without post-30 day health issues that necessitated pain management. The utility of ciprofloxacin as a radiation countermeasure required study in whole animals to understand the complex physiologic processing of the treatments. As ciprofloxacin MAY be utilized in a terrorist attack with radiation it was important to evaluate the irradiated non-human primate responses as a prelude to possible application in irradiated personnel. Thus, because of the complex interactions of ciprofloxacin in a whole irradiated animal, computer modeling, in vitro cell culturing, or evaluation of the published literature could not reveal the impact on the designated endpoints outlined in the IACUC approved protocol.

1274 mice

The research questions that we are attempting to address involve complex interactions between different tissues that would be affected by the administration of drugs to alleviate pain. The pain associated with the radiation experiments derives from the fact that the animals succumb to infections because of compromised immune systems. The pain and distress experienced by these animals would probably be similar to what we experience during severe cold and flu infections. Because of the physiological complexity of radiation injury, and our lack of having a full understanding of its induction and progression, there is just no other way to evaluate the effectiveness of TS designed to enhance survival than to do survival experiments using intact animals.

56 mice

As described in the protocol sections on "Non-animal Alternatives considered" and "Anesthesia/Analgesia/Tranquilization", the research questions that we are attempting to address involve complex interactions between different tissues that would be affected by the administration of drugs to alleviate pain. Although all painful procedures such as injection of the tumor cells and irradiation will be done under anesthesia, animals may experience discomfort and pain as a result of the postirradiation tissue injury and tumor growth. Pain arising out of the postirradiation sequelae and tumor growth cannot be alleviated since it may interfere with the objective of the study. The hypothesis of the protocol, viz., preferential protection of normal tissue by TT during irradiation of prostate tumor is based on the assumption of the differential distribution of TT in favor of the normal tissue. Administration of analgesics may affect the partitioning of TT between normal and tumor tissue and the results derived may not be conclusive. Influence of drugs and other factors on permeation of other drugs has been reported earlier (11,12). Since postirradiation sequelae and tumor growth may cause pain and discomfort, which will not be alleviated with analgesics, all mice other than unirradiated controls will be under the unalleviated pain category (E).

2944 mice

The use sedatives and analgesics will be used with a degree of caution and on an individual animal-in-need basis. Reasons for our proposed limited use of sedatives and analgesics only in cases of severe pain/discomfort are as follows: (1) in mice, clinical signs of minimal or even moderate pain/discomfort have the potential to interfere with the identification of clinical signs and alter the hematological and survival responses of the treated animals, and (b) the use and analgesics and anesthetics on an individual basis might interfere with basic functional elements of the irradiated animal's innate and acquired immune system. In this regard for example, there is a wealth of information that clearly documents the effect of sedatives and opiates on neutrophil production and function.

224 mice

Irradiated animals die due to compromised immune response and microbial infections. Percentage of surviving animals is indicator of radioprotective ability of any radioprotectant. There is no other way to know radioprotective ability of any agent. We have also discussed that we cannot give anesthetic agent to animals since they interact with immune system, and in turn affect result of survival studies.

90 mice

This study is divided into two study groups with only a small percentage of animals (16%) in this protocol in the unalleviated pain category. The first study group is the leukemia chemoprevention group consisting of 180 animals. Of this group 135 are control group animals (no radiation + no drug; No radiation, No Cells, etc). These animals are not expected to develop leukemia within the time period of the experiment (12 months from initiation of experiment); age-related leukemia may occur in animals older than 16 months; the study animals will be euthanized at or before 12 months so no age-related leukemia is expected in this group or study. The second study group is the serial euthanasia assessment groups consisting of 660 animals. Of this group 570 are control group animals (no radiation, no drug, no cells, etc) and are not expected to develop leukemia. This leaves 135 animals that will develop leukemia under the induction conditions; although 105 of those animals are receiving the nontoxic chemo-treatments which are expected to decrease the number of animals actually developing leukemia. Nevertheless, 135 animals may develop leukemia. This leukemogenesis could cause more than momentary unalleviated pain or distress since there are no known studies which specifically detail pain in murine leukemia development. As I indicated in the previous section regarding the pain search, human studies of leukemia development do not indicate that pain or distress is a noticeable symptom of leukemia development nor are there any definitive data in mice regarding pain and leukemia. To decrease any pain or suffering, after the cell injection animals will be monitored 3x weekly for 8 weeks and thereafter daily until they are euthanized. In this protocol we are using a narrow range in weight loss or gain (10%) as a parameter for euthanasia. These animals cannot be given any prophylactic pain medication for two reasons. 1) There is no specific information indicating what dose of analgesic would be effective to alleviate the discomfort/distress/pain of leukemia development. 2) Prophylactic analgesia could be contraindicated and detrimental to the protocol purpose. During leukemia development the immune system is affected and the injection of anti-inflammatory agents could affect its development.